

## LEDER-GIEMSA STAIN FOR BONE MARROW TREPINE – A TECHNICAL REPORT

Ş.K. Cheong MBBS, MRCP and S.M. Chong MBBS, MRCPATH

Department of Pathology, Faculty of Medicine, National University of Malaysia, Kuala Lumpur.

### Summary

**Leder** and Giemsa stains were combined on decalcified bone marrow trephine sections obtained by the Jamshidi needle. This method was prospectively carried out on 39 consecutive bone marrow trephine sections in parallel with the haematoxylin and eosin (H & E) stain. We found that the Leder-Giemsa stain enabled us to readily identify myeloblasts, cells of the neutrophilic series, mast cells, plasma cells, osteoblasts and megaloblasts. It appears to be a useful adjunct to the routine study of bone marrow sections with **H&E** stain as it can be performed satisfactorily on formalin-fixed decalcified bone marrow trephine sections.

Keywords: Leder-Giemsa stain, Bone marrow trephine.

### INTRODUCTION

Bone marrow biopsy may be carried out using Jamshidi needle and formalin-fixed decalcified sections routinely stained with haematoxylin and eosin. Giemsa and **Leder** stains of separate sections have been used to aid in the study of bone marrow.<sup>1,2</sup> We have successfully combined both stains on the same section and evaluated this method on 39 consecutive cases. The objective of this paper is to share our experience with this procedure.

### METHODS AND MATERIALS

Bone marrow trephine sections between 1st November 1984 and 30th July 1985 received by the Histopathology laboratories were studied using the Leder-Giemsa (LG) stain in parallel with the H & E stain. The methods and materials are as follows:

#### Leder stock solutions

- 4% pararosaniline hydrochloride:  
pararosaniline hydrochloride 1.0 gm  
distilled water 20.0 ml  
hydrochloric acid, concentrated 5.0 ml  
  
(Gently warm solution after mixing, filter and refrigerate at 4°C).
- 4% sodium nitrite solution:  
sodium nitrite 2.0 gm  
distilled water 50.0 ml
- Michaelis veronal acetate buffer stock solution:  
sodium acetate 4.857 gm  
sodium diethylbarbiturate 7.357 gm  
distilled water 250.0 ml.

- Michaelis veronal acetate buffer working solution (pH 7.42):  
veronal acetate buffer stock solution 42.4 ml  
0.1 N hydrochloric acid 37.6 ml
- Esterase substrate solution:**  
naphthol AS-D chloroacetate 20.0 mg  
N,N dimethylformamide 2.0 ml  
(prepare just before use)

#### Leder staining solution

Mix 0.1 ml of 4% pararosaniline and 0.1 ml of 4% sodium nitrite. Wait for 60 seconds until straw colour develops. Add 60.0 ml veronal buffer solution with pH 7.42 to the mixture. Adjust pH to 6.3 with 1.0 N HCl. About 5 drops would be required. Add 2.0 ml esterase substrate solution. Mix, and then filter. Solution should be milky pink before filtration, light pink after. Staining solution is now ready for use.

#### Giemsa staining solution

standard giemsa stain	2.0 ml
buffered distilled water (pH 6.8)	48.0 ml

#### Procedure of Leder-Giemsa stain

- Deparaffinize and hydrate to distilled water.
- Incubate at room temperature (about 25°C) for 35 minutes in **Leder** staining solution.
- Rinse well with distilled water, 3 changes.
- Immerse in Giemsa staining solution OVERNIGHT.

Address for reprint requests: Dr. S.K. Cheong, Department of Pathology, Faculty of Medicine, National University of Malaya, P. O. Box 12418, 50778 Kuala Lumpur.

5. Rinse with distilled water.
6. Wash with 0.5% acetic acid until the section is pink. About 2 minutes would be required.
7. Rinse under running tap water.
8. Dehydrate rapidly and clear with xylol.
9. Mount in DPX (distrene, dibutyl phthlate, xylene).

Source of Chemicals

Chemicals and reagents were obtained from the following commercial sources:

Naphthol AS-D chloroacetate	Sigma Chemical Co. USA
<b>Pararosaniline</b>	Sigma Chemical Co. USA
N, N <b>dimethyl</b> formamide	Sigma Chemical Co. USA
Giemsa	Difco Laboratories, UK

A review was carried out to consider aspects in which the LG stain enhanced the study of the bone marrow.

RESULTS AND DISCUSSION

The types and number of cases studied are shown in Table 1. The diagnosis **was** based on bone marrow findings.

The LG stain was found to be useful in the study of the following:

Myeloid: Erythroid Ratio (M: E Ratio)

In histological sections, the granulocytic (myeloid) to nucleated red cells (erythroid) ratio varies from 2:1 to 3:1. A change in ratio may indicate hyperplasia or hypoplasia of a cell type with or without changes of overall cellularity. The LG stain was particularly suitable for this assessment, because granules of cells of the granulocytic series stain scarlet whereas the cytoplasm of normoblasts stain purple.

The Red Cell Series

With **H&E**, normoblasts exhibit round nuclei and perinuclear halo. The nuclei appear pyknotic. In megaloblastic erythropoiesis, the red cell precursors appear larger with relative nuclear pallor. They are easily confused with myeloblasts of acute myeloid leukaemia. The LG stain helped to identify megaloblasts more readily as these exhibit deep violet **rims** of **cytoplasm** and large pale nuclei with a fine chromatin pattern.

TABLE 1

TYPE AND NUMBER OF CASES STUDIED

Conditions	No. of Cases
Acute lymphoblastic leukaemia	5
Acute myeloid leukaemia	1
Acute leukaemia (undifferentiated)	1
Chronic myeloid leukaemia	1
Myelofibrosis	1
Aplastic anaemia	3
Granulocytic hypoplasia	1
Megaloblastic erythropoiesis	1
Metastatic tumour	1
No definitive diagnosis	7
Inadequate tissue	17

The differential staining of the various marrow elements is summarized in Table 2. Figs. 1 to 4 illustrate some of the staining effects in four different haematological disorders. The **Granulocytic** Series and the Mast Cells

**Eosinophils** appear bright orange and are easily identified in H&E stained sections. Neutrophilic granulocytes are identified by their nuclear shapes. Neutrophils, band forms and metamyelocytes are easily recognised. However, myelocytes, promyelocytes and myeloblasts are difficult to identify with certainty.

In LG stained sections, eosinophils appear brownish. The cytoplasm of the cells of the **neutrophilic** series, from myeloblasts to neutrophils, stains scarlet. Mast cells stain intensely in a similar way rendering the nuclei not recognisable. Neutrophilic and mast cell granules stain scarlet due to the presence of an esterase.

The LG stain appears particularly useful in demonstrating acute myeloblastic leukaemia, granulocytic hyperplasia or hypoplasia, and mast cell hyperplasia.

**Lymphocytes, Plasma Cells and Osteoblasts**

In **H & E** stained sections, it is **sometimes** difficult to distinguish between normoblasts

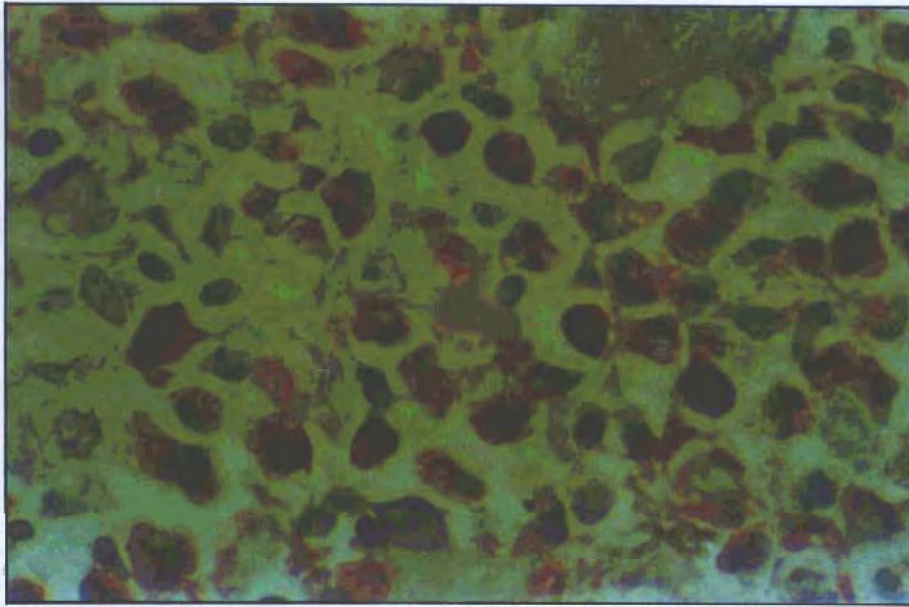


FIG. 1 : Chronic myeloid leukemia. The cells of the neutrophilic series stain scarlet. LG stain x 1000.

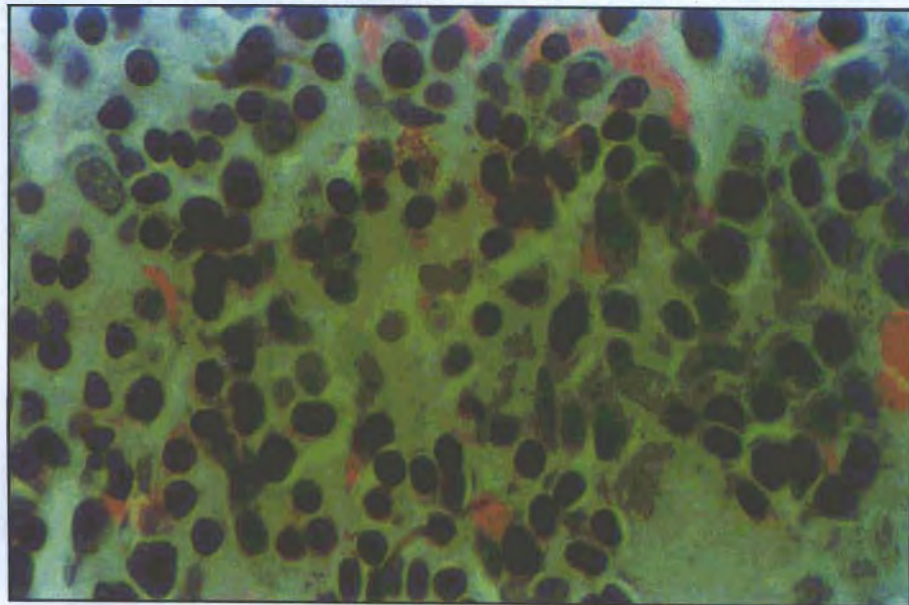


FIG. 2 : Granulocytic hypoplasia. The section shows conspicuous absence of scarlet-staining neutrophilic series. LG stain x 1000.

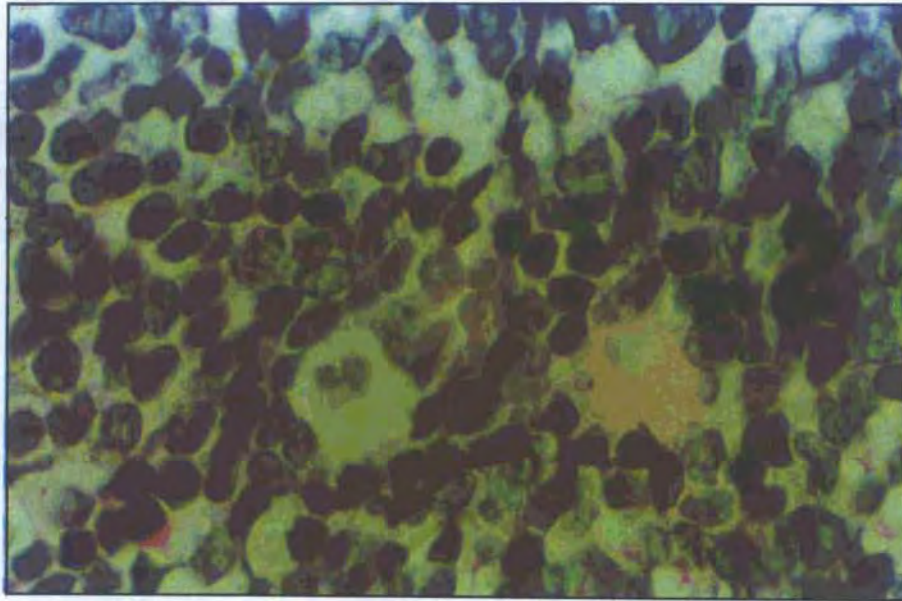


FIG. 3 : Acute lymphoblastic leukaemia. The lymphoblasts do not show scarlet staining. LG stain x 1000.

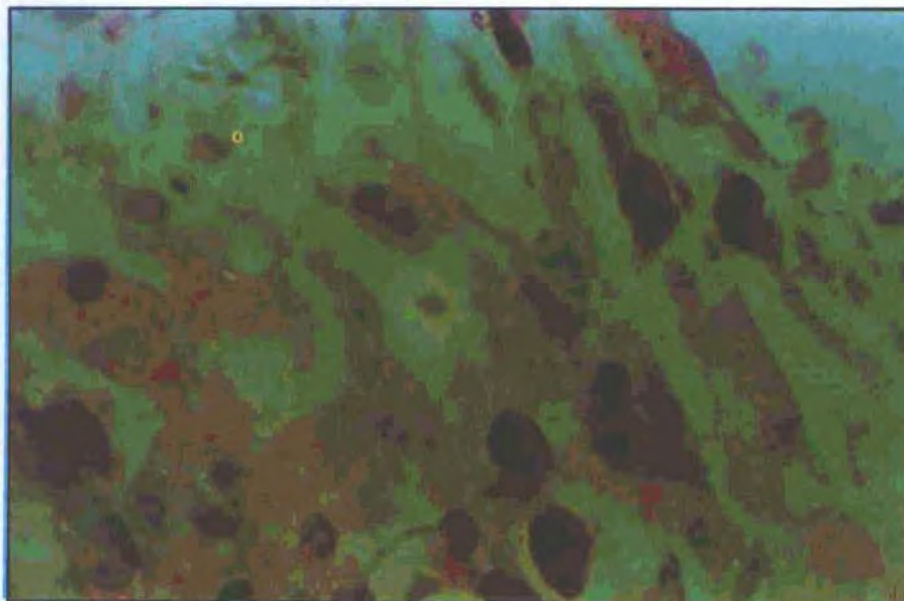


FIG. 4 : Aplastic anaemia. Mast cells show intense scarlet staining. LG stain x 1000.

TABLE 2  
STAINING REACTION  
OF THE **LEDER-GIEMSA** STAIN

Cell or Tissue	Colour Reaction
Neutrophilic granules	Scarlet
Mast cell granules	Scarlet
Eosinophilic granules	Brown
<b>Plasma</b> cell cytoplasm	Violet
Osteoblast cytoplasm	Violet
Megaloblast cytoplasm	Violet
Nuclei	Blue
Collagen and bone	Pink
Cartilage	Violet
Red cells	Pink

and lymphocytes. In general, lymphocytes have pale staining nuclei showing more nuclear details. They do not exhibit artificial perinuclear halo. Such a distinction was better seen in LG stained sections in which the lymphocyte

#### REFERENCES

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nuclei showed distinctive fine chromatin pattern in contrast to the coarse chromatin condensation of the normoblast nuclei.

The LG stain also accentuated plasma cells which showed a prominent pale golgi zone, violet cytoplasm and an eccentric nucleus. Osteoblast cytoplasm stained violet and was easily identified due to sharp contrast to the pink coloured bone.

#### Haematological Malignancy

In diffuse infiltration of bone marrow by leukaemic blasts and lymphoma cells, it is difficult to distinguish one from the other in H&E stained histological sections. The LG stain helped to identify the myeloid leukaemias. It was particularly useful in acute myelomonocytic leukemia where two populations of blast cells can be identified in the bone marrow, myeloblasts staining scarlet and monoblasts violet. It was not useful in distinguishing acute lymphoblastic leukaemia from non-Hodgkin's lymphoma.

We have found that the Leder-Giemsa stain enabled us to easily a) recognise acute myeloid leukaemia, b) identify cells of the neutrophilic series, c) identify mast cells, plasma cells and osteoblasts, and d) distinguish megaloblasts from leukaemic blasts and lymphoma cells. It appears to be a useful adjunct to the routine study of bone marrow sections as it can be satisfactorily carried out on formalin-fixed decalcified bone marrow obtained by the Jamshidi needle.

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